

Live Vaccine Strain *Francisella tularensis* Is Detectable at the Inoculation Site but Not in Blood after Vaccination against Tularemia

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Introduction. Live vaccine strain (LVS) *Francisella tularensis* is a live, attenuated investigational tularemia vaccine that has been used by the US Army for decades to protect laboratory workers. Postvaccination bacterial kinetic characteristics of LVS at the inoculation site and in the blood are unknown and, therefore, were assessed in a prospective study. LVS vaccination of laboratory workers provided the opportunity to compare culture with polymerase chain reaction (PCR) for the detection of *F. tularensis* in human clinical samples.

Methods. Blood and skin swab samples were prospectively collected from volunteers who received the LVS tularemia vaccine at baseline (negative controls) and at 5 specified time points (days 1, 2, 7 or 8, 14 or 15, and 35 after vaccination). Bacterial culture and PCR of whole blood samples (17 volunteers) and inoculation site swabs (41 volunteers) were performed.

Results. The culture and PCR results of all blood samples were negative. Results of real-time PCR from the inoculation site samples were positive for 41 (100%) of 41 volunteers on day 1, for 40 (97.6%) of 41 volunteers on day 2, for 24 (58.5%) of 41 on day 7 or 8, for 6 (16.7%) of 36 on day 14 or 15, and for 0 (0%) of 9 on day 35. Positive results of bacterial cultures of the inoculation site samples occurred significantly less frequently, compared with PCR testing, with 4 (9.8%) of 41 volunteers having positive results on day 1 ($P < .001$) and 4 (9.8%) of 41 on day 2 ($P < .001$); all results from subsequent days were negative.

Conclusions. *F. tularensis* LVS genomic DNA was detected in the majority of samples from the inoculation site up to 1 week after LVS vaccination, with real-time PCR being more sensitive than culture. Our data suggest that bacteremia does not occur after LVS vaccination in normal, healthy human volunteers.

Many experts believe that *Francisella tularensis* may pose a significant threat if it is used as a biological weapon [1]. Although antibiotics may be effective at treating naturally acquired infection, certain segments of the population (e.g., military personnel and civilian first responders) may benefit from the protection provided by vaccination in an intentional use scenario. This is especially pertinent when considering the risk of use of an antibiotic-resistant organism. A strain of atten-

uated *F. tularensis*, later named the live vaccine strain (LVS), was given to the United States by the Russian Federation (formerly the Union of Soviet Socialist Republics) in the 1950s as part of a scientific exchange program [1]. The incidence of tularemia among laboratory workers at Fort Detrick, Maryland, declined substantially after vaccination was instituted [2]. In the Fort Detrick experience with LVS vaccination, no cases of person-to-person transmission have been observed. The vaccine is administered by scarification and forms a small lesion, mimicking a very mild case of ulceroglandular tularemia.

Two major concerns accompany the administration of any live, attenuated vaccine. There is a risk of dissemination in the host leading to systemic or remote infection, and there is a risk of person-to-person transmission. Although person-to-person transmission does not occur with naturally acquired tularemia [1], there

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14. ABSTRACT Introduction. Live vaccine strain (LVS) Francisella tularensis is a live, attenuated investigational tularemia vaccine that has been used by the US Army for decades to protect laboratory workers. Postvaccination bacterial kinetic characteristics of LVS at the inoculation site and in the blood are unknown and, therefore, were assessed in a prospective study. LVS vaccination of laboratory workers provided the opportunity to compare culture with polymerase chain reaction (PCR) for the detection of F. tularensis in human clinical samples. Methods. Blood and skin swab samples were prospectively collected from volunteers who received the LVS tularemia vaccine at baseline (negative controls) and at 5 specified time points (days 1, 2, 7 or 8, 14 or 15, and 35 after vaccination). Bacterial culture and PCR of whole blood samples (17 volunteers) and inoculation site swabs (41 volunteers) were performed. Results. The culture and PCR results of all blood samples were negative. Results of real-time PCR from the inoculation site samples were positive for 41 (100%) of 41 volunteers on day 1, for 40 (97.6%) of 41 volunteers on day 2, for 24 (58.5%) of 41 on day 7 or 8, for 6 (16.7%) of 36 on day 14 or 15, and for 0 (0%) of 9 on day 35. Positive results of bacterial cultures of the inoculation site samples occurred significantly less frequently, compared with PCR testing, with 4 (9.8%) of 41 volunteers having positive results on day 1 (P<.001) and 4 (9.8%) of 41 on day 2 (P<.001); all results from subsequent days were negative. Conclusions. F. tularensis LVS genomic DNA was detected in the majority of samples from the inoculation site up to 1 week after LVS vaccination, with real-time PCR being more sensitive than culture. Our data suggest that bacteremia does not occur after LVS vaccination in normal, healthy human volunteers.		
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is always a theoretical concern with live vaccines, especially in the context of potentially immunocompromised contacts. Assessment of these risks begins with a determination of the presence and distribution of the agent after inoculation. There is a particular concern with tularemia vaccine, because it is applied to the skin surface with scarification, in a similar manner to the vaccinia (smallpox) vaccine, with subsequent development of cutaneous lesions. No previous study has made use of multiple diagnostic platforms to detect the presence of LVS *F. tularensis* at the scarification site or in the blood after vaccination, or whether persistence of bacteria relates to clinical or immune responses to vaccination.

The LVS vaccine offers a unique opportunity to compare the utility of diagnostic techniques for tularemia. Multiple PCR platforms have been developed to detect *F. tularensis* [3–8] and have been tested for accuracy of ulceroglandular tularemia diagnosis [3, 9, 10]. However, the sensitivity of PCR from whole blood samples is decreased, perhaps because of the presence of inhibitors in human blood [11]. *F. tularensis* is difficult to grow from human clinical specimens and requires special conditions, such as a CO₂-enriched atmosphere and supplementation of media with cysteine, to support its growth [11]. *F. tularensis* has been isolated in culture of samples from the site of infection with ulceroglandular disease, but rarely from blood samples [12, 13]. Performing a blind subculture on agar plates for negative blood culture bottles may improve the yield of detecting *F. tularensis* [12].

The purpose of this study was (1) to test for the presence of bacteria at the inoculation site and in whole blood samples after LVS vaccination; (2) to compare the use of real-time PCR with bacterial culture techniques for the detection of LVS *F. tularensis*; and (3) to determine associations between the detection of bacteria and the clinical and serological response to vaccination. To accomplish these objectives, we conducted a prospective, observational study with volunteers who received the LVS tularemia vaccine at the US Army Medical Research Institute of Infectious Diseases (Fort Detrick, Maryland).

METHODS

Volunteers. Volunteers were enrolled from the Special Immunizations Program at the US Army Medical Research Institute of Infectious Diseases. In this program, personnel are offered vaccination against potential laboratory-acquired infections. Per Special Immunizations Program procedures, immunocompromised personnel were excluded from vaccination. Personnel were also excluded if they had prior evidence of immunity to tularemia (defined by the Special Immunizations Program as a serum microagglutination titer >1:40), or if they were taking antibiotics ≤7 days before vaccination. Volunteers were recruited to participate in our clinical protocol after agreeing to receive the LVS vaccination, and informed consent was

obtained. The protocol was approved by the US Army Medical Research Institute of Infectious Diseases Human Use Committee (FY04-16, 30 June 2004).

LVS tularemia vaccine. The vaccine was administered by scarification. A single 0.6-mL drop of LVS vaccine (2×10^9 colony-forming units [cfu] per mL) was placed on the volar surface of the left forearm. A bifurcated needle was used to administer 15 pricks through the drop. The skin surface was then cleaned with sterile gauze, and all excess fluid was removed. Volunteers were given no special bandage or covering for the site. There were no restrictions on showering or wetting the vaccination site.

Follow-up and sample collection. Volunteers presented for follow-up evaluation of their inoculation site and symptom screening on days 1, 2, 7 or 8, and 14 or 15 after vaccination. Samples were taken at these time points. The presence or absence of fever (defined as a temperature >38°C) or lymphadenopathy, lesion description, and surrounding erythema were recorded at each visit. A “take” reaction was defined as the appearance of an erythematous papule, vesicle, or eschar. Volunteers were queried regarding symptoms of fatigue, malaise, or headache. Antibody response to vaccination was measured by microagglutination titer at 28 days after vaccination, and titers were reevaluated at day 56 for volunteers who had a negative serological response at day 28.

Volunteers were enrolled from 2 iterations of LVS vaccination. From the first iteration, 17 volunteers were enrolled; 10 mL of blood and inoculation site samples were obtained from each person. Blood specimens were divided immediately; 2 mL were frozen at –70°C for subsequent real-time PCR analysis, and 8 mL were inoculated directly into blood culture bottles (BD Bactec Plus Aerobic/F; Becton Dickinson).

Baseline skin swabs from this group were performed on the volar surface of the left forearm. Postvaccination swabs were performed directly at the site of inoculation. Four different personnel obtained skin swabs using similar techniques, which involved applying the swab to the center of the inoculation site, rolling the swab, then making concentric circles up to 2–3 cm away from the center. At each collection date, a Dacron swab (Puritan) for PCR was performed first and was immediately placed into 500 µL of PBS (Sigma) containing 0.3% Tween-20 (Sigma) and was frozen at –70°C, as described elsewhere [14]. A second swab was performed then placed in a transport media system (BBL CultureSwab Plus; Becton Dickinson). The bacterial swabs were plated onto modified Thayer-Martin agar (Remel) within 3–4 h. In a subset of 9 volunteers who were available in this initial group of 17, skin swab samples were also obtained on day 35.

In the second iteration of vaccination, swabs were obtained from 24 volunteers using the same time points, but no blood samples were obtained. Swabs were obtained in the following

order: a Dacron swab was used for real-time PCR testing, a cotton/polyester swab was used for immediate plating unto modified Thayer Martin agar, and a third swab was used for laboratory plating.

PCR platform. The real-time PCR platform, which has been described elsewhere [15], has 100% sensitivity for 62 replicates at a concentration of 50 fg and 100% specificity against a panel of 60 organisms. The limit of detection for this platform was 5000 cfu/mL for frozen skin swab samples and 250 cfu/mL for frozen whole blood samples (M.J.H., S.R.C., D. A. Kulesh, D.A.N., and M.P.U.; unpublished data). Briefly, primers and probes for 2 different gene sequences (*tul4* and *fopA*) were designed, optimized, and tested using the TaqMan-MGB chemistry tests on the Ruggedized Advanced Pathogen Identification Device real-time PCR machines (Idaho Technology). Samples were extracted using Qiagen DNA mini kits (Qiagen). All PCR samples were tested in triplicate with internal positive controls [16]. A sample was classified as positive if the results of 2 of 3 tested samples were positive using both assays.

A standard curve was used to convert crossing threshold values from the skin swab samples into approximate colony-forming unit counts. This standard curve was generated using a series of swab samples that were inoculated with a known quantity of LVS bacteria (NDBR 101 Lot #4), frozen, then extracted as described above.

Bacterial culture. Skin swab samples were plated onto modified Thayer-Martin agar, which contains antibiotics to inhibit normal skin flora, supports the growth of LVS (tested in our laboratory), and has been used in previous studies of ulceroglandular tularemia [10, 17]. Culture plates were incubated in 10% CO₂ at 37°C and were read 5–7 days after plating. Gram staining was performed on all growth on culture plates. Gram-positive organisms were excluded from further analysis. Gram-negative coccobacilli, with the characteristic appearance and gray color of colonies, were confirmed as LVS with PCR testing (using the *tul4* and *fopA* assays, as described above [15]). Other gram-negative organisms were identified using Vitek and API bacterial identification systems (BioMérieux) to exclude the possibility of *F. tularensis*.

All blood culture bottles were incubated in the Bactec 9050 blood culture machine (Becton Dickinson). Bottles were incubated for 28 days, and terminal subculture was performed by removing 100 µL from the blood culture bottle and plating it on glucose cysteine agar plates (Remel). Plates were incubated in 10% CO₂ at 37°C and read after 5–7 days.

Statistical analysis. The primary outcome variable was the frequency of positive skin swab samples at each time point by either culture or PCR. We assumed that the percentage of positive results at crucial time points would be ~10%, and we sought to minimize our 95% CI to ~10% (prevalence, 10%;

95% CI, 0%–20%). A sample size of 40 volunteers was selected to meet these conditions.

All binary variables were compared using Fisher's exact test. When comparing interval or continuous variables with a normal distribution, *t* tests were used. Postvaccination titers were analyzed as a continuous variable. Postvaccination titers were log₁₀ transformed after adding 1 to each titer to offset for 0. After testing was performed, the final titer results were described as geometric mean titers. When describing the correlation between 2 interval variables with a normal distribution, a Pearson's product moment coefficient was calculated. Statistical significance was defined as *P* < .05 for all tests.

RESULTS

A total of 28 (68%) of 41 volunteers were male, with a median age of 38 years (range, 22–59 years). The volunteers' clinical characteristics are included in table 1. One volunteer recorded a temperature of 38.3°C on day 2. One volunteer developed 4 × 5-cm axillary lymphadenopathy 28 days after vaccination. No other volunteers had fever or lymphadenopathy. All volunteers had a take reaction. No severe erythema or deep ulcer was observed in any volunteer. During this study, there were no incidences of inadvertent inoculation of remote sites or person-to-person transmission. All volunteers had samples obtained at each of the 5 time points, except for 1 volunteer who missed the day-14 or 15 sample collection. Four of the skin swab samples obtained on day 14 or 15 for PCR were excluded from analysis, because both the samples and the extraction-positive controls for those 4 samples were negative, indicating a failure in the extraction procedure for that set of samples. The positive controls for the extraction of the other 36 samples on day 14 or 15 (and on all other days) were positive.

The results of blood cultures, including terminal subcultures, were negative at baseline and at all time points. Additionally, PCR results of whole blood samples were negative using both *tul4* and *fopA* assays at baseline and at all time points.

Baseline skin swab samples were negative for LVS *F. tularensis*

Table 1. Clinical characteristics of volunteers.

Characteristic	Value
Prevaccination titer detected, no. (%) of volunteers ^a	2 (5) ^b
Postvaccination symptoms, no. (%) of volunteers ^a	
Fatigue or malaise ^c	8 (20)
Headache ^c	10 (24)
Day 1 erythema, maximum width in mm (range)	5 (0–20)
Day 2 erythema, maximum width in mm (range)	12 (0–30)

^a *n* = 41.

^b These 2 volunteers had prevaccination titers of 1:20 and 1:40.

^c Symptoms of fatigue and/or malaise, as well as headache, were considered to be present if volunteer noted that symptom at any visit during the first 14 days after vaccination.

by both real-time PCR assays. Skin swab samples were uniformly positive for LVS *F. tularensis* by PCR on day one, 40 (97.6%) of 41 samples were positive on day 2, and several samples were positive 1 and 2 weeks after vaccination (table 2). Two volunteers had negative results on day 7 or 8 but positive results on day 14 or 15. All 9 samples from day 35 were negative with both PCR assays. The quantitative amount of LVS present with the 2 assays for positive samples is displayed in figure 1.

Bacterial cultures from the inoculation site were infrequently positive on the first 2 days after vaccination and were consistently negative after day 2 (table 2). Growth was positive for 2 volunteers on days 1 and 2. When culture techniques were compared (immediate bedside plating vs. use of transport media and plating) for the second group of 24 volunteers, the results were similar, as the positive cultures from day 1 (for 1 [4.2%] of 24 volunteers) and day 2 (for 2 [8.3%] of 24 volunteers) were positive using both techniques. Immediate inoculation had a mean colony-forming unit count of 4.6, whereas, when the transport medium was used, the mean colony-forming unit count was 2.1. Each sample that was culture-positive for LVS *F. tularensis* had the characteristic colony morphology and characteristic Gram stain pattern and was positive 3 of 3 times for both PCR assays for identification.

Results of the day-28 postvaccination serological testing are shown in figure 2. One volunteer did not experience seroconversion, with negative titers at 28 and 56 days postvaccination. There was no significant correlation between the detection of LVS bacteria at the inoculation site for any follow-up day and the day-28 postvaccination titer.

Detection of LVS at the vaccination site correlated with local inflammation. Volunteers with a positive PCR result at day 7 or 8 had greater erythema on day 2 (14 mm vs. 8 mm; $P = .05$). A positive PCR result at day 7 or 8 or at day 14 or 15 was not associated with increased frequency of systemic symptoms. There was no significant correlation between detecting LVS at the inoculation site and age or sex.

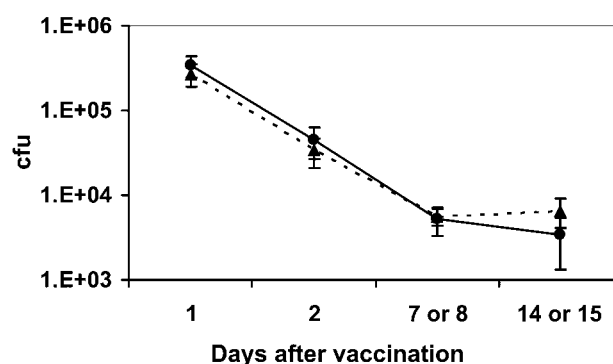


Figure 1. Live vaccine strain of *Francisella tularensis* bacteria at the inoculation site, as measured by real-time PCR on a log₁₀ scale. The real-time PCR assays are represented as follows: *tul4*, by the solid line; *fopA*, by the dashed line. Equivocal samples were not included. Error bars, SE. cfu, colony-forming unit.

DISCUSSION

In this study, we demonstrated that LVS genomic DNA is almost universally detectable at the inoculation site for the first 2 days after LVS vaccination and is detectable in a significant number of volunteers for 1–2 weeks after vaccination. Live LVS bacteria were detected in culture for the first 2 days after vaccination. While LVS DNA at the inoculation site was detected well past 2 days, this does not confirm the presence of viable bacteria past this time point. However, we suspect that genome detection reflects the presence of viable organisms. Volunteers generally left the inoculation sites uncovered and are required to shower at least daily when working in biocontainment suites. Therefore, one would assume that LVS DNA would not persist at the inoculation site in the absence of actively replicating organisms. The presence of this bacteria did not cause person-to-person transmission in our study, which is analogous to clinical tularemia infection in which person-to-person transmission does not occur [11].

We found no correlation between detection of LVS at the vaccination site and postvaccination systemic symptoms such

Table 2. PCR and culture results for swab samples from the inoculation site.

Postvaccination time point	PCR		Culture		P
	No. of samples with positive results/no. tested (%)	95% CI	No. of samples with positive results/no. tested (%)	95% CI	
Day 1	41/41 (100)	...	4/41 (10)	3%–23%	<.001
Day 2	40/41 (98)	87%–100%	4/41 (10)	3%–23%	<.001
Day 7 or 8	24/41 (59)	42%–74%	0/41 (0)	0%–9%	<.001
Day 14 or 15	6/36 (17)	6%–33%	0/40 (0)	0%–9%	.009
Day 35	0/9 (0)	0%–34%	0/9 (0)	0%–34%	

NOTE. P values were calculated using Fisher's exact test.

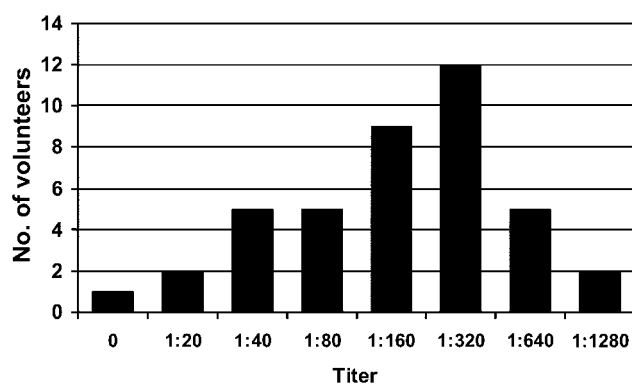


Figure 2. Day-28 postvaccination titers to the live vaccine strain *Francisella tularensis* vaccine. Titers were measured by microagglutination.

as fever, malaise, and headache. Only a small number of volunteers experienced such symptoms, and the study was not powered to detect such an association. PCR detection of LVS at day 7 or 8 was associated with increased erythema at day 2. However, as previously mentioned, day-28 serological responses were not significantly higher among volunteers with positive PCR results, compared with volunteers with negative PCR results on day 7 or 8.

The results of this study suggest that real-time PCR is a more-sensitive diagnostic modality, compared with culture, for the detection of *F. tularensis* from skin swabs. The challenges of growing *F. tularensis* in culture-based systems are well documented [11]. Inoculating agar plates at the bedside immediately after swabbing did not improve the frequency of detection of LVS bacteria. Prior clinical studies of ulceroglandular tularemia indicate that PCR is more sensitive than culture, although the differences were not as substantial as those observed in our study [10, 17, 18]. Our study presented the unique occasion to directly compare 2 diagnostic modalities under controlled conditions. All participants were tested at the same time points after infection and had negative baseline test results. We attempted to ensure that all volunteers received approximately the same inoculum by using standardized vaccination administration methods. Our results imply that real-time PCR may be a useful clinical diagnostic modality for ulceroglandular tularemia, especially as this technology becomes increasingly available.

Culture and PCR of whole blood samples after LVS tularemia vaccination revealed no evidence of bacteremia at any time point. Naturally acquired tularemia is rarely diagnosed by blood culture, and the limit of detection for *F. tularensis* with PCR on whole blood tends to be high because of inhibitors [11–13]. However, in vitro experiments in our laboratory suggested that amounts as low as 4 colony-forming units could be detected in the Bactec blood culture system (data not shown). In

this study, 8 mL of blood was inoculated into the blood culture bottles. If volunteers were bacteremic, it is, therefore, likely that the concentration of organisms was <1 cfu/mL. Alternatively, volunteers could have developed a transient bacteremia at a time point that was not measured in our study.

We acknowledge some potential limitations of this study. Although swab sampling methods were standardized among personnel, it is possible that different techniques, even with the same person, could result in differences in the bacterial yield, which is relevant to the quantification of bacterial DNA. Swabs for PCR were consistently obtained before bacterial culture swabs in our study. Although not all bacteria would be removed from a site after a single swabbing of the area, the order of obtaining swabs may have given real-time PCR detection a slight advantage. The frequency of detection of LVS by bacterial culture of both blood samples and swabs of the inoculation site was nonexistent or low, respectively, and it is possible that the results would be improved by increasing the volume of blood collected or optimizing the media for swab cultures. However, we intentionally used commercially available media to allow for ease of comparison with future studies and to resemble available media for the practicing clinician. Additionally, we were unable to determine whether DNA detected from the inoculation site swabs was intracellular or extracellular, which would have revealed interesting information regarding the host response to this vaccine.

The LVS tularemia vaccine does not appear to produce bacteremia, but LVS genomic DNA is detectable at the inoculation site up to 2 weeks after inoculation. The persistence of LVS DNA after inoculation suggests, but does not confirm, that replicating organisms are present at the inoculation site, which poses a theoretical risk of person-to-person transmission. However, this risk of transmission is likely to be very minimal, especially given the epidemiology of natural *F. tularensis* infection. Real-time PCR may be superior to culture for the detection of LVS bacteria after vaccination, with the extrapolation being that real-time PCR offers a preferable modality for the diagnosis of tularemia from skin lesions. Further study exploring the relationship of bacteria at the inoculation site and local and systemic immunologic response is needed.

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References

1. Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon: medical and public health management. *JAMA* 2001;285:2763–73.

2. Burke DS. Immunization against tularemia: analysis of the effectiveness of live *Francisella tularensis* vaccine in prevention of laboratory-acquired tularemia. *J Infect Dis* **1977**; 135:55–60.
3. Emanuel PA, Bell R, Dang JL, et al. Detection of *Francisella tularensis* within infected mouse tissues by using a hand-held PCR thermocycler. *J Clin Microbiol* **2003**; 41:689–93.
4. Fulop M, Leslie D, Titball R. A rapid, highly sensitive method for the detection of *Francisella tularensis* in clinical samples using the polymerase chain reaction. *Am J Trop Med Hyg* **1996**; 54:364–6.
5. Grunow R, Spletstoeser W, McDonald S, et al. Detection of *Francisella tularensis* in biological specimens using a capture enzyme-linked immunosorbent assay, an immunochromatographic handheld assay, and a PCR. *Clin Diagn Lab Immunol* **2000**; 7:86–90.
6. Higgins JA, Hubalek Z, Halouzka J, et al. Detection of *Francisella tularensis* in infected mammals and vectors using a probe-based polymerase chain reaction. *Am J Trop Med Hyg* **2000**; 62:310–8.
7. Junhui Z, Ruifu Y, Jianchun L, et al. Detection of *Francisella tularensis* by the polymerase chain reaction. *J Med Microbiol* **1996**; 45:477–82.
8. Karhukorpi EK, Karhukorpi J. Rapid laboratory diagnosis of ulceroglandular tularemia with polymerase chain reaction. *Scand J Infect Dis* **2001**; 33:383–5.
9. Johansson A, Ibrahim A, Goransson I, et al. Evaluation of PCR-based methods for discrimination of *Francisella* species and subspecies and development of a specific PCR that distinguishes the two major subspecies of *Francisella tularensis*. *J Clin Microbiol* **2000**; 38:4180–5.
10. Sjostedt A, Eriksson U, Berglund L, Tarnvik A. Detection of *Francisella tularensis* in ulcers of patients with tularemia by PCR. *J Clin Microbiol* **1997**; 35:1045–8.
11. Ellis J, Oyston PC, Green M, Titball RW. Tularemia. *Clin Microbiol Rev* **2002**; 15:631–46.
12. Provenza JM, Klotz SA, Penn RL. Isolation of *Francisella tularensis* from blood. *J Clin Microbiol* **1986**; 24:453–5.
13. Reary BW, Klotz SA. Enhancing recovery of *Francisella tularensis* from blood. *Diagn Microbiol Infect Dis* **1988**; 11:117–9.
14. Klote MM, Ludwig GV, Ulrich MP, et al. Absence of oropharyngeal vaccinia virus after vaccinia (smallpox) vaccination. *Ann Allergy Asthma Immunol* **2005**; 94:682–5.
15. Christensen DR, Hartman LJ, Loveless BM, et al. Detection of biological threat agents by real-time PCR: comparison of assay performance on the RAPID, the LightCycler, and the Smart Cycler platforms. *Clin Chem* **2006**; 52:141–5.
16. Hartman LJ, Coyne SR, Norwood DA. Development of a novel internal positive control for Taqman-based assays. *Mol Cell Probes* **2005**; 19: 51–9.
17. Johansson A, Berglund L, Eriksson U, et al. Comparative analysis of PCR versus culture for diagnosis of ulceroglandular tularemia. *J Clin Microbiol* **2000**; 38:22–6.
18. Dolan SA, Dommaraju CB, DeGuzman GB. Detection of *Francisella tularensis* in clinical specimens by use of polymerase chain reaction. *Clin Infect Dis* **1998**; 26:764–5.